spoligotyping and mycobaterial interspersed repetitive unit (MIRU) typing. Beijing ancient strains and Harrlem strains predominated among aborigines, while Beijing modern strains were common among veterans and the general population. All Beijing strains were further analyzed by typing the NTF loci and RD deletion. Results suggest a chronological trend among Beijing isolates from the three groups: isolates from the aborigines had signatures compatible with ancient lineages, and those from veterans and the general population were more contemporary. Our data indicate that the distribution of MTB genotypes/strains in Taiwan is associated with different populations whose migratory activities occurred between 55 and 500 years ago. These results suggest that transmission of MTB may have been relatively restricted to close contacts.
Part II study: A total of 356 MTB isolates were analyzed for the genotypes of MTB in metropolitan city. Major spoligotypes found were Beijing lineages (52.5%), followed by Haarlem lineages (13.5%) and EAI plus EAI-like lineages (11%). When MIRU-VNTR was employed, 140 patterns were identified, including 36 clusters by 252 isolates and 104 unique patterns, and the largest cluster comprised 95 isolates from the Beijing family. The combination of spoligotyping and MIRU-VNTR revealed that 236 (67%) of the 356 isolates were clustered in 43 genotypes, and thus the proportion of TB infection that can be considered due to the recent transmission is estimated (236–43)/356 = 54%. Patients infected with Beijing strains were younger than those with other strains. Moreover, infected persons younger than 25 years had Beijing modern stain, suggesting a possible recent spread in the young population by this family of TB strain in Taipei.
Conclusion: The distribution of MTB in Taiwan is closely associated with the ethnicity and migratory population at different historical periods. The high clustering of MTB isolates and the high prevalence of Beijing family strains in young persons strongly indicate that a stringent control policy should be taken in Taiwan, particularly in metropolitan city.

I-38 Recent advances in the diagnosis of TB

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Microscopy is still the backbone and benchmark of laboratory diagnostics in TB, but it is well recognized that better technical approaches are urgently needed to overcome limitations in test sensitivity, time to result and ease of use. The ultimate alternative products are expected to provide rapid testing in primary care settings. To this end a number of opportunities are being followed up, ranging from identification of novel biomarkers for various stages of disease to the development of enabling technologies suited for implementation in the different sectors of the health care system in developing countries, i.e., from the reference laboratory down to the health post in rural areas where the biggest gaps exist.
It has become widely accepted that resolution of open issues in TB diagnostics can only be achieved by systematic and novel research approaches. Among these are the seroprofiling of the whole *M. tuberculosis* proteome using antigen arrays, discovery of marker molecules in sputum and urine employing most advanced mass spectrometry. Both development programs are progressing at fast pace.
In addition, two rapid molecular detection systems have been developed and are close to entering international evaluation studies: an assay developed by Eiken Chemicals represents the first manual molecular TB test emplyoing rapid DNA amplification based on LAMP technology whereas the GeneXpert platform from Cepheid, Inc. provides fully automated point of care Mtb DNA detection by rtPCR at an ultrasensitive level. These technology approaches are considered breakthrough diagnostics that for the first time will enable robust Mtb nucleic acid detection in fast and affordable formats.
The recent introduction and WHO endorsement of technologies such as liquid culture, rapid speciation and molecular DST demonstrate that our current approaches are effective and already show their impact. These achievements will be furter complemented by the introduction of iLED, a low cost fluorescence microscope that will significantly incraese sensitivity of sputum smear analysis.
However, it is also clear that those achievements – all based on private-public-partnerships – were only made possible by implementing a strong industry-type quality and project management system as an ultimate prerequisite for successful product development. Using available, leading edge tools in biomarker discovery, detection technology and project management, we are now in an excellent position to further narrow and even close current gaps in TB diagnostics.

I-39 Molecular diagnosis of tuberculosis

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Molecular tests have brought a significant impact on laboratory diagnosis of tuberculosis (TB) from simple detection of *Mycobacterium tuberculosis* from clinical specimens to rapid detection of MDR/XDR-TB. As an effort to implement those tests to clinical mycobacteriology laboratories, we also have been working on developing and evaluation various molecular techniques using clinical samples and mycobacterial cultures.
Firstly, we tried to detect *M. tuberculosis*-specific DNA in sputum samples by polymerase chain reaction (PCR), and target DNAs were IS6110, region of deletions (RDs), and the *rpoB* gene, etc. Since the proportion of non-tuberculous mycobacteria (NTM) has increased in many countries, we also developed molecular tests, in which primer sets detecting both *M. tuberculosis* and NTMs were included and evaluated them using sputum samples and mycobacterial cultures from both solid and liquid culture systems.
Secondly, we developed a series of molecular tests for identification of *Mycobacterium* species. Initially, we employed a PCR-restriction enzyme analysis (PRA) targeting a polymorphic region of the *rpoB* gene. Using the PRA, we were able to differentiate more than 50 *Mycobacterium* species. *Mycobacterium* species-specific probes were then designed and used in a dot-blot assay and a reverse-blot hybridization assay (RBHA). At least 13 *Mycobacterium* species, covering more than 95% of mycobacteria from clinical samples in Korea, were successfully identified either by dot-blot or by RBHA.
Thirdly, we explored the dot-blot assay and RBHA to detect mutations of genes associated with resistance to anti-TB drugs, both the first and second line drugs. As appeared in numerous literatures, we found the in-house made RBHA is working properly for detecting mutations at *katG*, *inhA*, and *ahpC* genes for resistance to INH, at the *rpoB* gene to RIF, at the *rrs* and *rpsL* genes to SM, KAN, AMK, and CAP, and the *gyrA* gene to OFX. Although there is still a substantial portion of *M. tuberculosis* isolates which do not have mutations at these genes, RBHA has been useful in reducing turn-around time substantially for rapid detection

of anti-TB drug resistance both from smear-positive sputum samples and cultures. Recently, we developed RBHAs for detection of mutations at the *gyrA* gene and at the *rrs* and *rpsL* genes, which are responsible for XDR-TB.
Lastly, we developed an RBHA-based dual test for TB/NTM identification and detection of mutations at the *rpoB* gene, which are the most critical information of MDR-TB using one set of primers. This was possible because the polymorphic regions for *Mycobacterium* species identification and for mutations involved in resistance to RIF are located closed within 360 bp.
Throughout our exercises in evaluating these molecular diagnostic tests, we found advantages and problems in implementing them to the clinical mycobacteriology laboratories, and these points will be discussed with numerous examples.

I-40 Detection of *Mycobacterium tuberculosis* infection using interferon-gamma release assay in Shenzhen, China

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Background: Tuberculosis (TB) remains one of the major public health problems worldwide, with 95% of cases and 98% of deaths occurring in developing countries. According to the epidemiological surveillance carried out in 2000, approximately 550 million individuals in China have been infected with Mtb. based on the result of TST. Nevertheless, TST has been shown inferior to Interferon-Gamma Release Assay (IGRA) in terms of the specificity and sensitivity as reported in countries with low incidence of tuberculosis. Therefore, both the diagnostic value of IGRA in the setting of china and the number of Mtb. infection determined by IGRA warrant investigation.
Objectives: (1) To evaluate the sensitivity and specificity of an in-house Mtb. specific IFN-γ Elispot kit in diagnosis of Mtb. infection. (2) To investigate the prevalence of Mtb. infection in different population using the in-house Elispot kit.
Methods: By screening the IFN-γ response to different antigen proteins (eg. recombinant ESAT-6 protein) and individual peptides derived from Mtb. antigens, an in-house IFN-γ Elispot kit was developed. The sensitivity and specificity of the in-house Elispot kit in diagnosis of tuberculosis were evaluated in paralleled comparison with commercial Quantiferon-TB-Gold. The prevalence of Mtb. infection in different healthy populations in Shenzhen was investigated using the in-house Elispot kit and compared with TST.
Results: The sensitivity and specificity of in-house Elispot kit for diagnosis of tuberculosis were 86% and 92%, respectively, both of which are comparable with the commercial Quantiferon-TB-Gold and significantly higher than TST. In addition, the price of in-house Elispot kit was only about 1/4 of commercial kits such as Qunatiferon-TB-Gold or T.SPOT.TB. Using the Elispot assay, the prevalence of Mtb. infection in healthy population varied from 5.88% to 55.6% depends on their exposure to active tuberculosis cases.
Conclusion: An in-house affordable IGRA kit for diagnosis of Mtb. infection has been developed with sensitivity and specificity comparable to that of commercial kits. The prevalence of Mtb. infection in healthy population determined by IGRA is significantly lower than that of TST.

Concurrent Session 7 – Understanding HCV

I-41 Pathogenesis and clinical impact of insulin resistance in chronic HCV infection

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Insulin resistance (IR) is more often seen in chronic hepatitis virus C infection than in other forms of chronic liver disease. Recent research indicates that IR seen in patients chronically infected with HCV may be directly caused or at least favoured by the virus itself. In the transgenic mice model the HCV core protein has been shown to induce IR as a consequence of increased TNF production. Other mechanisms have been also proposed, based on the activation of a number of cytokines, and on generation of oxidative stress, as consequence of the direct effect of the HCV and of HCV proteins on mitochondria and on the hepatocyte endoplasmic reticulum. Recent results obtained in our laboratory implicate endogenous interferon production in HCV related IR due to common intracellular signalling shared by interferon and insulin.
IR has a number of clinical consequences in hepatitis C. It associates with more rapid progression of fibrosis and with reduced response to antiviral therapy with interferon or peghilated-interferon and ribavirin. The mechanisms by which IR promotes fibrosis progression in the liver of patients with HCV include: development of hepatic steatosis, hyperleptinemia, increased TNF production and reduce expression of PPARγ receptors. These concepts have practical implications for the management of patients with HCV, and diet and exercise aimed to reduce metabolic abnormalities should be recommended, particularly in overweighted cases.
Several studies have clearly demonstrated that sustained virological response to interferon-based therapy is significantly reduced in patients with HCV and high Homeostasis Model of Assessment (HOMA) IR index. This effect is independent of presence of type 2 diabetes and of obesity and is already evident with HOMA values >3. We have recently shown that baseline hyperinsulinemia has inhibitory effects on serum HCV-RNA decay seen as early as 24 hours after the first injection of pegylated-interferon, indicating direct downregulation by insulin of intracellular IFN signalling. The impairment of the early antiviral response correlate with reduces sustained response to PEG-IFN plus ribavirin treatment. Several studies have now confirmed reduced SVR in patients with IR, independently of the HCV genotype. Interestingly patients with SVR experienced a significant reduction in the HOMA index that returned within the normal range at the time of HCV clearance, suggesting a direct relationship between HCV replication and IR.
Obviously IR seen in patients with HCV is not always related to direct effects of HCV and may also occur as consequence of the classical metabolic syndrome frequently seen in the general population, particularly in the Western civilized countries. However, also in these cases IR is associated with more progressive liver disease and reduces response to antiviral therapy and should be therefore corrected when possible.
Ongoing studies are assessing the role of insulin sensitisers (metformine, pioglitazone) for improving the response to antiviral therapy in HCV patients with IR, but results so far reported have been controversial and inconclusive.